molbank

MDPI

Communication

Synthesis and Characterization of New Potential Hypoxia-Sensitive Azo-thiacalix[4]arenes Derivatives

Farida B. Gabdrakhmanova [1,*], Ekaterina S. Churbanova [2], Mohamed A. Khalifa [2,3], Sofia R. Kleshnina [1], Svetlana E. Solovieva [1,2] and Igor S. Antipin [1,2]

[1] Arbuzov Institute of Organic and Physical Chemistry, FRC Kazan Scientific Center, Russian Academy of Sciences, Arbuzova 8, 420088 Kazan, Russia
[2] Department of Organic and Medical Chemistry, Kazan Federal University, Kremlevskaya 18, 420008 Kazan, Russia
[3] Chemistry Department, Faculty of Science, Damanhour University, Damanhur 22511, Egypt
* Correspondence: kleo-w@mail.ru

Abstract: The subject of this article is new potential hypoxia-sensitive azo-thiacalix[4]arenes derivatives in the 1,3-alternate configuration. Previously, it was shown that azo derivatives of calix[4]arene in the cone conformation form complexes with rhodamine dyes. The present work is devoted to the synthesis of new azo derivatives using the thiacalix[4]arene platform. A new highly productive method for the synthesis of thiacalixarene with four anionic sulfonate azo fragments on the lower rim (compounds **2a**–**b**) for further complexation with the most common cationic dyes is reported. The chemical structures of the products obtained were established based on 1H and 13C NMR, IR spectroscopy, MALDI TOF mass spectrometry, and elemental analysis.

Keywords: thiacalix[4]arene; azo derivatives; hypoxia sensing

Citation: Gabdrakhmanova, F.B.; Churbanova, E.S.; Khalifa, M.A.; Kleshnina, S.R.; Solovieva, S.E.; Antipin, I.S. Synthesis and Characterization of New Potential Hypoxia-Sensitive Azo-thiacalix[4]arenes Derivatives. *Molbank* **2023**, *2023*, M1570. https://doi.org/10.3390/M1570

Academic Editor: R. Alan Aitken

Received: 29 December 2022
Revised: 23 January 2023
Accepted: 28 January 2023
Published: 31 January 2023

1. Introduction

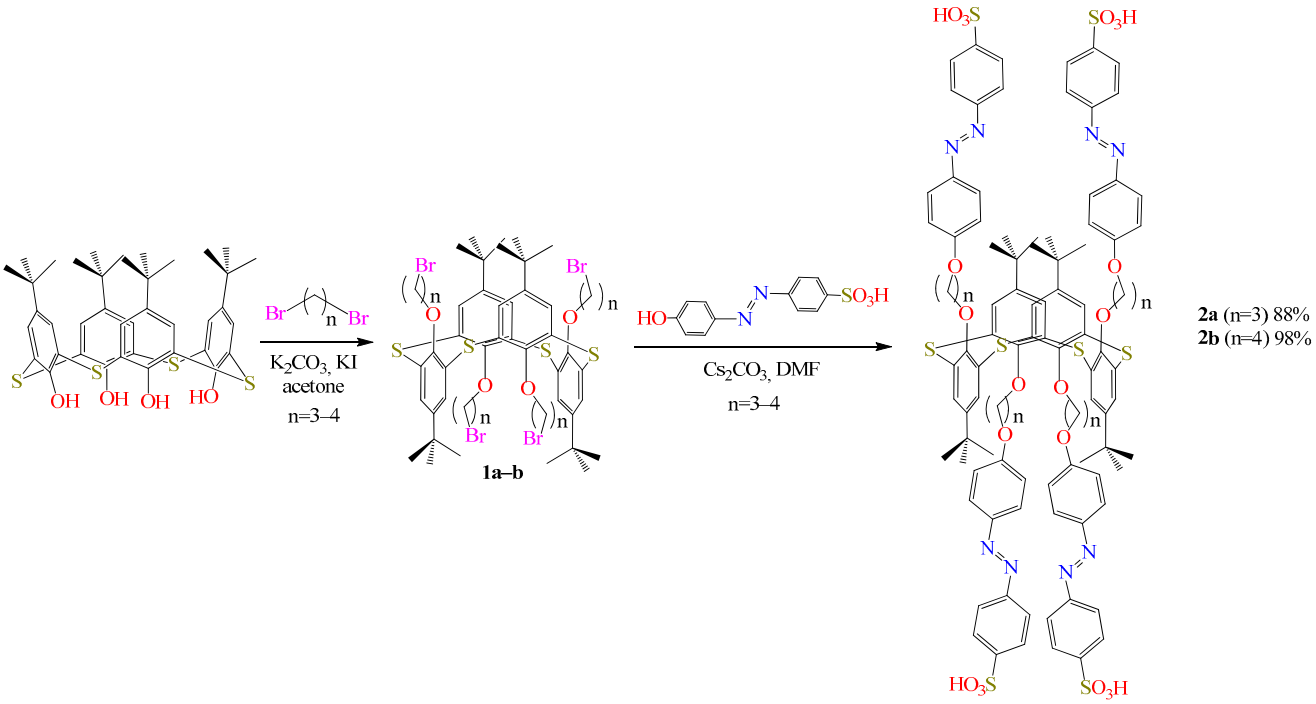
Hypoxia is a pathological process that develops as a result of an insufficient oxygen supply to tissues or a violation of its use by tissues [1,2]. It accompanies many diseases and pathophysiological states in the human body [3,4]. Particularly, hypoxia can be regarded as an indicator of tumor aggressiveness [5]. For this reason, there is a great interest in developing hypoxia visualization tools. Changes in the vascular system and blood flow resulting from tumor growth lead to the appearance of rapidly growing tumors of hypoxic regions, which in turn is accompanied by an increase in reductase activity [6]. The susceptibility of the azo bond to reduction by azoreductase formed the basis for the development of methods for visualizing hypoxia [7–11]. In this regard, the creation of hypoxia-sensitive systems based on complexes of azo derivatives of calixarenes seems promising [12]. Previously, it was shown that the calix[4]arenes with anionic carboxyl and sulfonate azo fragments in the *cone* configuration form complexes with rhodamine dyes [13]. The resulting complexes do not luminesce, but luminescence is recovered under hypoxic conditions. The present work is devoted to the synthesis of thiacalix[4]arenes **2a**–**b** with anionic sulfonate azo groups in the *1,3-alternate* conformation for further complexation with cationic dyes and the creation of signaling systems based on them for detecting hypoxia.

2. Results and Discussion

We synthesized new O-substituted thiacalix[4]arenes **2a**–**b** in the *1,3-alternate* conformation containing azo fragments linked by methylene spacers of different lengths (three and four units) (Scheme 1).

The synthesis of the initial bromopropyl thiacalixarene derivative **1a**–**b** [14] as well as 4-[4-hydroxyphenylazo]-benzenesulfonic acid [15] was carried out using methods from the literature. We synthesized new derivatives of tetra-azo-thiacalix[4]arene **2a**–**b** in *1,3-alternate* form from the reaction of **1a**–**b** with azo fragments. Compounds **2a**–**b** were obtained after

refluxing the reaction mixture for 5 days with 88% and 98% yield, respectively. Azo derivatives **2a**–**b** were obtained as a result of the nucleophilic substitution reaction of the OH-nucleophile of the phenolic azo derivative with haloalkyl-thiacalixarene **1a**–**b** in a basic medium.



Scheme 1. Synthetic pathway for thiacalixarenes **2a**–**b**.

The structures of **2a**–**b** were decisively assigned based on IR, ^{1}H and ^{13}C NMR spectroscopy, MALDI TOF mass spectrometry, and elemental analysis. The ^{1}H NMR spectrum of 25,26,27,28-tetrakis-4-[4-(4′-sulfonylphenyldiazinyl)phenoxy]butoxy-5,11,17,23-tetra(*tert*-butyl)thiacalix[4]arene **2b** displayed four aromatic protons as two apparent doublet and two singlet signals at δ 7.88, 7.79, 7.41, and 7.07 and four methylene protons as multiplet signals at δ 3.96, 3.91, 1.67, and 1.34 in addition to one methyl proton signal at δ 1.24 ppm. Additionally, the substitution reaction was confirmed by the ^{13}C NMR spectrum that showed the four sp^3-methylene carbons at δ 69.63, 68.98, 36.77, and 34.91 (see Figures S1–S8).

3. Materials and Methods

The NMR spectra were recorded on Bruker Avance 400 and 500 Nanobay (Bruker Corporation, Billerica, MA, USA) instruments with signals from residual protons of DMSO-*d6* solvent as the internal standard. MALDI mass spectra were obtained using an UltraFlex III TOF/TOF spectrometer in the linear mode; *p*-nitroaniline or 2,5-dihydroxybenzoic acid was used as the matrix. Elemental analysis was performed using a PerkinElmer PE 2400 CHNS/O analyzer.

All reagents and solvents were purchased from either Acros (Geel, Belgium) or Sigma-Aldrich (Burlington, VT, USA) and used without further purification. 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrakis-[(3-bromopropoxy)]2,8,14,20-tetrathiacalix[4]arene **1a**, 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrakis-(4-bromobutoxy)-2,8,14,20-tetrathiacalix[4]arene **1b** [14], and 4-[4-hydroxyphenylazo]-benzenesulfonic acid [15] were synthesized according to reported methods.

Synthesis of azo-thiacalix[4]arenes **2a**–**b**.

25,26,27,28-Tetrakis(*p*-bromalkoxy)-5,11,17,23-tetra(*tert*-butyl)thiacalix[4]arene **1a**–**b** (0.1 mmol) in DMF (10 mL) was added to 4-[4′-hydroxyphenylazo]benzenesulfonic acid (0.6 mmol) and Cs_2CO_3 (1 mmol). The reaction mixture was heated to 85 °C for 5 days.

Then, the solvent was removed under vacuum; water was added to the residue. The precipitate formed was filtered off, washed with water, and dried in the air.

25,26,27,28-tetrakis-3-[4-(4′-sulfonylphenyldiazinyl)phenoxy]propoxy-5,11,17,23-tetra (*tert*-butyl)thiacalix[4]arene **2a**.

Yield 88 %, orange powder, mp > 360 °C. R_f (ethyl acetate:hexane = 10:1) 0.26. IR: 1440 (N=N); 1191; 1143; 1120; 1006 (SO_2). ^{1}H NMR (400 MHz, DMSO-*d6*, 25 °C) δ, ppm: 7.90 (app d, 8H, Ar_{azo}-H), 7.79 (s, 16H, Ar_{azo}-H), 7.46 (s, 8H, ArH), 7.09 (app d, 8H, ArH_{azo}-H), 3.98–4.07 (m, 16H, OCH_2), 1.49 (m, 8H, CH_2), 1.23 (s, 36H, *t*-Bu). ^{13}C NMR (101 MHz, DMSO-*d6*, 25 °C): δ 163.32, 162.34, 157.16, 152.73, 151.26, 147.19, 146.96, 128.35, 128.13, 127.66, 127.23, 125.70, 122.74, 120.79, 116.00, 66.08, 36.78.78, 35.00, 35.78, 35.00, 35.00. 31.80, 29.28. Mass spectrum MALDI (DHB), *m/z*: 2028.4 $[M - 4H + K]^+$. Found, %: C 58.79; H 5.71; N 6.45. $C_{100}H_{104}N_8O_{20}S_8 \cdot 3H_2O \cdot 2(CH_3)_2NC(O)H$. Calculated, %: C 58.87; H 5.78; N 6.48.

25,26,27,28-tetrakis-4-[4-(4′-sulfonylphenyl-diazinyl)phenoxy]butoxy-5,11,17,23-tetra (*tert*-butyl)thiacalix[4]arene **2b**.

Yield 98 %, orange powder, mp > 360 °C. R_f (ethyl acetate:hexane = 10:1) 0.56. IR: 1440 (N=N); 1237; 1194; 1120; 1007 (SO_2). ^{1}H NMR (500 MHz, DMSO-*d6*, 25 °C) δ, ppm: 7.88 (app d, 8H, Ar_{azo}-H), 7.79 (s, 16H, Ar_{azo}-H), 7.41 (s, 8H, ArH), 7.07 (app d, 8H, ArH_{azo}-H), 3.91–3.96 (m, 16H, OCH_2), 1.67 (m, 8H, CH_2), 1.34 (m, 8H, CH_2), 1.24 (s, 36H, *t*-Bu). ^{13}C NMR (101 MHz, DMSO-*d6*, 25 °C): δ 163.31, 162.41, 157.84, 152.69, 151.29, 147.18, 146.43, 128.95, 128.71, 127.65, 125.64, 122.73, 115.92, 69.63, 68.98, 36.77, 34.91, 31.95, 31.78. Mass spectrum MALDI (*p*-NA), *m/z*: 2145.5 $[M + H]^+$. Found, %: C 60.95; H 5.60; N 6.05. $C_{104}H_{112}N_8O_{20}S_8 \cdot (CH_3)_2NC(O)H$. Calculated, %: C 60.98; H 5.69; N 5.98.

4. Conclusions

Thus, we have demonstrated the synthesis of thiacalix[4]arenes **2a**–**b** with anionic sulfonate azo groups in the *1,3-alternate* conformation in high yields. The chemical structures of the products obtained were established based on ^{1}H and ^{13}C NMR, IR spectroscopy, MALDI TOF mass spectrometry, and elemental analysis.

Supplementary Materials: The following supporting information can be downloaded. Figure S1: ^{1}H NMR spectrum of compound **2a** (DMSO-*d6*, 40 MHz, 25 °C); Figure S2: ^{13}C NMR spectrum of compound **2a** (DMSO-*d6*, 101 MHz, 25 °C); Figure S3: MALDI TOF mass spectrum of compound **2a** (DHB); Figure S4: IR spectrum of compound **2a** (KBr tablet); Figure S5: ^{1}H NMR spectrum of compound **2b** (DMSO-*d6*, 500 MHz, 25 °C); Figure S6: ^{13}C NMR spectrum of compound **2b** (DMSO-*d6*, 101 MHz, 25 °C); Figure S7: MALDI TOF mass spectrum of compound **2b** (p-NA); Figure S8: IR spectrum of compound **2b** (KBr tablet).

Author Contributions: Conceptualization, F.B.G. and S.E.S.; investigation, M.A.K., E.S.C. and S.R.K.; data curation, F.B.G., M.A.K. and S.R.K.; writing—original draft preparation, F.B.G.; writing—review and editing, F.B.G. and S.E.S.; supervision, S.E.S. and I.S.A.; project administration, F.B.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Russian Science Foundation, grant number 22-73-00138.

Data Availability Statement: The data presented in this study are contained within the article or in the Supplementary Materials or are available on request from the corresponding author Farida Gabdrakhmanova.

Acknowledgments: The authors are grateful to the Assigned Spectral-Analytical Center of Shared Facilities for Study of Structure, Composition and Properties of Substances and Materials of the Federal Research Center of Kazan Scientific Center of Russian Academy of Sciences (CSF-SAC FRC KSC RAS) for technical support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bristow, R.G.; Hill, R.P. Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability. *Nat. Rev. Cancer* **2008**, *8*, 180–192. [CrossRef] [PubMed]
2. Brown, J.M.; Wilson, W.R. Exploiting tumour hypoxia in cancer treatment. *Nat. Rev. Cancer* **2004**, *4*, 437–447. [CrossRef] [PubMed]
3. Lee, J.W.; Ko, J.; Ju, C.; Eltzschig, H.K. Hypoxia signaling in human diseases and therapeutic targets. *Exp. Mol. Med.* **2019**, *51*, 1–13. [CrossRef] [PubMed]
4. Schellinger, I.N.; Cordasic, N.; Panesar, J.; Buchholz, B.; Jacobi, J.; Hartner, A.; Klanke, B.; Jakubiczka-Smorag, J.; Burzlaff, N.; Heinze, E.; et al. Hypoxia inducible factor stabilization improves defective ischemia-induced angiogenesis in a rodent model of chronic kidney disease. *Kidney Int.* **2017**, *91*, 616–627. [CrossRef] [PubMed]
5. Span, P.N.; Bussink, J.B. Biology of Hypoxia. *WB Saunders* **2015**, *45*, 101–109. [CrossRef] [PubMed]
6. Zhou, H.; Qin, F.; Chen, C. Designing Hypoxia-Responsive Nanotheranostic Agents for Tumor Imaging and Therapy. *Adv. Healthc. Mater.* **2021**, *10*, 2001277. [CrossRef] [PubMed]
7. Chevalier, A.; Mercier, C.; Saurel, L.; Orenga, S.; Renard, P.Y.; Romieu, A. The first latent green fluorophores for the detection of azoreductase activity in bacterial cultures. *Chem. Commun.* **2013**, *49*, 8815–8817. [CrossRef] [PubMed]
8. An, Y.; Chen, C.; Zhu, J.; Dwivedi, P.; Zhao, Y.; Wang, Z. Hypoxia-induced activity loss of a photo-responsive microtubule inhibitor azobenzene combretastatin A4. *Front. Chem. Sci. Eng.* **2020**, *14*, 880–888. [CrossRef]
9. Wang, C.; Zhang, S.; Huang, J.; Cui, L.; Hu, J.; Tan, S. Novel designed azo substituted semi-cyanine fluorescent probe for cytochrome P450 reductase detection and hypoxia imaging in cancer cells. *RSC Adv.* **2019**, *9*, 21572–21577. [CrossRef] [PubMed]
10. Zhao, X.B.; Ha, W.; Gao, K.; Shi, Y.P. Precisely Traceable Drug Delivery of Azoreductase-Responsive Prodrug for Colon Targeting via Multimodal Imaging. *Anal. Chem.* **2020**, *92*, 9039–9047. [CrossRef] [PubMed]
11. Zhang, X.; Wu, M.; Li, J.; Lan, S.; Zeng, Y.; Liu, X.; Liu, J. Light-Enhanced Hypoxia-Response of Conjugated Polymer Nanocarrier for Successive Synergistic Photodynamic and Chemo-Therapy. *ACS Appl. Mater. Interfaces* **2018**, *10*, 21909–21919. [CrossRef] [PubMed]
12. Geng, W.-C.; Jia, S.; Zheng, Z.; Li, Z.; Ding, D.; Guo, D.-S. A noncovalent fluorescence turn-on strategy for hypoxia imaging. *Agewandte Chem. Int. Ed.* **2019**, *58*, 2377–2381. [CrossRef] [PubMed]
13. Mironova, D.; Burilov, B.; Galieva, F.; Khalifa, M.A.M.; Kleshnina, S.; Gazalieva, A.; Nugmanov, R.; Solovieva, S.; Antipin, I. Azocalix[4]arene-rhodamine supramolecular hypoxia-sensitive systems: A search for the best calixarene hosts and rhodamine guests. *Molecules* **2021**, *26*, 5451. [CrossRef] [PubMed]
14. Tyuftin, A.A.; Solovieva, S.E.; Muravrev, A.A.; Polyantsev, F.M.; Latypov, S.H.K.; Antipin, I. Synthesis and fluorescent properties of thiacalix[4]arenes containing terpyridyl fragments at the lower rim. *Chem. Bull. Int. Ed.* **2009**, *58*, 145–151. [CrossRef]
15. Wei, Y.; Zeng, Q.; Bai, S.; Wang, M.; Wang, L. Nanosized Difunctional Photo Responsive Magnetic Imprinting Polymer for Electrochemically Monitored Light-Driven Paracetamol Extraction. *ACS Appl. Mater. Interfaces* **2017**, *9*, 44114–44123. [CrossRef] [PubMed]